TITLE: "Baby Machine" Analysis of Cellular Gravity Sensitivity
GRANT NO: NAGW-4503
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DATES: 5/95-5/98

PROGRESS REPORT, 5/95-2/96

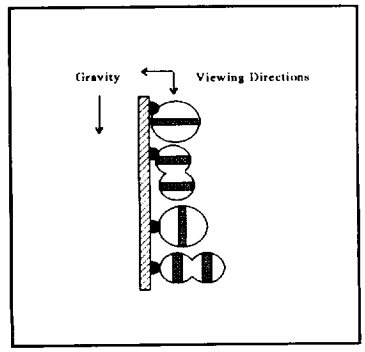
The overall goal of the project is to apply the "baby machine" culture technique to studies on the effects of gravity on cell growth, through analysis of effects on mitosis, growth rates, chromosome replication and segregation, and cellular senescence. The baby machine culture system has properties ideally suited for studies on the direct effects of gravity on cell growth and division. In this method cells are grown on tiny adhesive sites located on a nonadhesive surface such that each time a cell divides, one daughter cell remains attached to the surface while the other daughter is released. Thus, the cells are fixed in place so that they can be positioned precisely with respect to a fixed plane, and they do not spread on the surface. This culture system enables ground-based assessments of gravity-sensitive "windows" for any cell process by measuring the effects of the gravity vector, and long-term exposure to gravity averaging, on cell growth and division parameters. The specific aims for the first year are to design and construct modifications to the baby machine culture system for application to investigation of gravity sensitivity, and to use this modified culture system to evaluate the effects of gravity on the orientation of mitosis.

Preparation of "Baby Machine" culture flasks. A simple culturing technique was developed. The first step was to coat the bottoms of standard 50-ml polystyrene culture flasks with a nonadhesive surface. To achieve this, the flasks were first rinsed twice with methanol to remove loose bits of plastic and to pre-wet the surface. Then, polyhydroxyethylmethacrylate (PHEMA, Aldrich Chemicals) was dissolved in methanol at 5% (w/v) and volumes of 0.1 to 5.0 ml were pipetted into the flasks. After swirling to cover the bottom of the flasks, they were allowed to dry in a sterile hood. After drying for 24 hr or longer, small adhesive sites were placed on the PHEMA. The sites consisted of 2.8 or 4.5 μm-diameter tosylactivated Dynabeads obtained from Dynal Inc. The beads were either used directly or after surface modification. The modification involved resuspending the beads in 10 mg/ml acrylamide in 0.5 M borate buffer (pH 9.5) for 24 hr with gentle mixing. The beads were added to the PHEMA-coated flasks in PBS such that each flask contained approximately 2 x 10^6 beads dispersed randomly on the PHEMA surface. When modified beads were employed, the PBS buffer also contained 0.2% ammonium persulfate and 0.4% sodium metabisulfite in order to achieve a degree of covalent polymerization of the beads to the surface. After at least 2 days of incubation, the flasks were rinsed with PBS and used for cell attachment. A manuscript will be prepared shortly describing this technique for modification of culture flasks to

produce baby machines.

Analyses of baby machine culture properties. For addition of Chinese hamster ovary cells (CHO) to the baby machine flasks, approximately 1/2 of a confluent monolayer of CHO cells in a 50-ml culture flask in RPMI 1640 medium with 10% fetal calf serum was added to the flasks. The cells attached to the beads only, and within a few hours at least half of the beads contained an attached cell. At this point, or after essentially all beads contained cells, the unattached cells were poured off and fresh RPMI 1640 medium with 15% FCS was added and overlaid with 5% CO_2. The flasks were then placed at 37°C in a chamber surrounding the stage of an inverted microscope. The growth and division of the cells was followed with a video camera connected to a time-lapse VCR. Individual cultures were videotaped daily, and it could be seen that the cells were attached to the beads only such that upon subsequent growth and division, one daughter cell remained attached to the bead and the other was released. Growth in the culture flasks was generally maintained for about two weeks, until a specific analysis was completed. Growth was evaluated either in cultures which continued to multiply in the original medium or with flasks in which the daughter cells were removed by pouring off the medium and adding fresh medium. The growth and division of the cells was monitored using either 20X or 40X objectives such that there were from 10 to 100 cells, attached to beads, in the field recorded by the camera. At this cell density, mitosis was clearly evident to enable measurements of intermitotic times and orientation of mitosis. The following conclusions were reached as regards the behavior of the baby machine flasks and the growth properties of the cells. 1. Flasks coated with as little as 0.2 ml of 5% PHEMA completely prevented attachment of the cells to the bottom of the flask. 2. Surface-modified beads attached essentially permanently to PHEMA, which was not the case with unmodified beads. 3. Growth of the CHO cells in the baby machine flasks appeared to continue indefinitely, as long as fresh medium was added at intervals. 4. The interdivision times of the cells in the baby machine cultures was approximately 16 hr. 5. The doubling times of the cells appeared unaffected by the orientation of the flasks with respect to gravity.

Preliminary analysis of cellular gravity sensitivity. To begin the assessment of gravity sensitivity of mitosis, the flasks were oriented in different positions with respect to the gravity vector and the orientations of mitoses were observed by time-lapse videography. Most experiments to date have been performed with the baby machine culture flasks held vertically such that the gravity vector was parallel to the surface to which the beads were attached, as shown in the figure. This enabled measurement of the effect of gravity on the orientation of mitosis of cells suspended free in



space but fixed in place by attachment to a bead. Orientation of mitosis was determined by measuring the angle of the axis of mitosis relative to the vertical axis. Approximately 400 hrs of these tapes have been produced, and the video records are presently being analyzed. The analysis will require about two more months to complete, so only preliminary information is currently available. However, these preliminary analyses appear to suggest that neither growth rate nor the orientation of cellular mitosis are affected by the position of the gravity vector with respect to the cells. It must be emphasized that these conclusions are only preliminary, and the quantitation will have to be completed before reaching firm conclusions. Furthermore, the effects of gravity averaging on the rate and orientation of mitosis has yet to be determined.

Quantitation of the effects of the gravity vector on the orientation and frequency of mitosis should be completed by the end of the first year of the project. These findings will lead directly into the second aim of this project to determine the effects of gravity averaging on mitosis and the durations of the phases of the mitotic cycle. The preliminary stages of the latter project have been initiated. It requires pulse-labeling a baby machine culture with ^{3}H-thymidine and measuring the radioactivity in daughter cells as they are continuously released from the attached cell population. Techniques are currently underway to enable removal of the daughter cells at 2-hr intervals without disturbance of the immobilized population. The next step will be to perform these measurements on populations maintained in various positions with respect to gravity and simultaneously in flasks maintained in a clinostat to achieve gravity compensation.

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